CLAIMS

We claim:

1. A microorganism comprising a modified *pckA* gene.

2. The microorganism of Claim 1, wherein said *pckA* gene has been deleted.

3. The microorganism of Claim 1, wherein said *pckA* gene has been inactivated.

4. The microorganism of Claim 1, wherein said microorganism is a Gram-positive microorganism.

5. The microorganism of Claim 4, wherein said Gram-positive microorganism is a *Bacillus* species.

6. The microorganism of Claim 5, wherein said microorganism exhibits improved expression of at least one protein of interest, as compared to a microorganism having an unmodified *pckA* gene.

7. The microorganism of Claim 6, wherein at least one indigenous chromosomal region within said *Bacillus* species has been modified to produce a variant *Bacillus* strain and wherein said variant *Bacillus* strain expresses a higher level of said protein of interest as compared to said *Bacillus* species.

8. The microorganism of Claim 7, wherein said variant *Bacillus* strain comprises a heterologous polynucleotide encoding said protein of interest .

9. The variant *Bacillus* strain of Claim 7, wherein said variant *Bacillus* strain is *B. subtilis*.

10. The microorganism of Claim 7, wherein said at least one modified indigenous chromosomal region is selected from the group consisting of prophage regions, antimicrobial regions, regulator regions, multi-contiguous single gene regions, and operon regions.

11. The microorganism of Claim 10, wherein said at least one modified indigenous chromosomal region is selected from the group consisting of PBSX regions, skin regions, prophage 7 regions, SPβ regions, prophage 1 regions, prophage 2 regions, prophage 3 regions, prophage 4 regions, prophage 5 regions, prophage 6 regions, PPS regions, PKS regions, *yvfF-yveK* regions, DHB regions, and fragments thereof.

12. The microorganism of Claim 10, wherein said at least one modified indigenous chromosomal region is modified by deletion.

13. The microorganism of Claim 12, wherein said deletion comprises a deletion of one or more indigenous chromosomal regions or fragments thereof, wherein the indigenous chromosomal region includes about 0.5 to 500 kilobases.

14. An altered *Bacillus* strain comprising deletion of the *pckA* gene.

15. The altered Bacillus strain of Claim 14, wherein said altered *Bacillus* strain produces at least one protease.

16. The altered *Bacillus* strain of Claim 14, wherein said protease is subtilisin.

17. The subtilisin of Claim 16, wherein said subtilisin is selected from the group consisting of subtilisin 168, subtilisin BPN', subtilisin Carlsberg, subtilisin DY, subtilisin 147, subtilisin 309, and variants thereof.

18. The altered *Bacillus* strain of Claim 14, wherein said altered *Bacillus* strain further comprises at least one deletion in at least one chromosomal gene selected from the group of *sbo*, *slr*, *ybcO*, *csn*, *spoIISA*, *sigB*, *phrC*, *rapA*, *CssS*, *trpA*, *trpB*, *trpC*, *trpD*, *trpE*, *trpF*, *tdh/kbl*, *alsD*, *sigD*, *prpC*, *gapB*, *fbp*, *rocA*, *ycgN*, *ycgM*, *rocF*, and *rocD*.

20. The altered *Bacillus* strain of Claim 18, further comprising at least one mutation in at least one gene selected from the group consisting of *degU*, *degQ*, *degS*, *scoC4*, *spoIIE*, and *oppA*.

21. The altered *Bacillus* strain of Claim 20, wherein said mutation comprises *degU(Hy)32*.

22. The altered *Bacillus* strain of Claim 14, further comprising at least one DNA construct comprising an incoming sequence.

23. The altered *Bacillus* strain of Claim 22, wherein said incoming sequence comprises at least one selection marker and the *pckA* gene.

24. The altered *Bacillus* strain of Claim 23, wherein said incoming sequence further comprises at least one gene selected from the group consisting of *sbo*, *slr*, *ybcO*, *csn*, *spoIISA*, *sigB*, *phrC*, *rapA*, *CssS*, *trpA*, *trpB*, *trpC*, *trpD*, *trpE*, *trpF*, *tdh/kbl*, *alsD*, *sigD*, *prpC*, *gapB*, *fbp*, *rocA*, *ycgN*, *ycgM*, *rocF*, and *rocD*.

25. The altered *Bacillus* strain of Claim 24, wherein said at least one selection marker is located in between two fragments of said gene.

26. The altered *Bacillus* strain of Claim 22, wherein said incoming sequence comprises a selection marker and at least one homology box, wherein said homology box flanks the 5' and/or 3' end of said selection marker.

27. The altered *Bacillus* strain of Claim 22, wherein said incoming sequence is incorporated into the genome of said altered *Bacillus* strain.

28. An isolated nucleic acid comprising at least one sequence set forth in a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:37, SEQ ID NO:25, SEQ ID NO:21, SEQ ID NO:50, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:19, SEQ ID NO:31, SEQ ID NO:48, SEQ ID NO:46, SEQ ID NO:35, and SEQ ID NO:33.

29. An isolated nucleic acid sequence encoding an amino acid, wherein said amino acid is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:38, SEQ ID NO:26, SEQ ID NO:22, SEQ ID NO:57, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:55, SEQ ID NO:53, SEQ ID NO:36, and SEQ ID NO:34.

30. A purified DNA construct comprising an incoming sequence, wherein said incoming sequence comprises a selection marker and either a *cssS* gene, a *cssS* gene fragment or a homologous sequence thereof.

31. The DNA construct of Claim 30, wherein said selection marker is located between two fragments of said *cssS* gene.

32. The DNA construct of Claim 30, wherein said incoming sequence further comprises at least one homology box wherein the homology box flanks the 5' and/or 3' end of said selection marker.

33. The DNA construct of Claim 30, wherein said DNA sequence comprises at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:37, SEQ ID NO:25, SEQ ID NO:21, SEQ ID NO:50, SEQ ID NO:29, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:19, SEQ ID NO:31, SEQ ID NO:48, SEQ ID NO:46, SEQ ID NO:35, and SEQ ID NO:33.

34. The DNA construct of Claim 30, comprising at least one gene that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:38, SEQ ID NO:26, SEQ ID NO:22, SEQ ID NO:57, SEQ ID NO:30, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:55, SEQ ID NO:53, SEQ ID NO:36, and SEQ ID NO:34.

35. A host cell transformed with the DNA construct of Claim 30.

36. The host cell of Claim 35, wherein said host cell is selected from the group consisting of *Escherichia* and *Bacillus* cells.

37. The host cell of Claim 35, wherein said incoming sequence is chromosomally integrated in said host cell.

38. A method for enhancing production of at least one protein by a microorganism, comprising the steps:

a) providing a microorganism host cell;

b) inactivating the *pckA* gene in said host cell to produce an altered strain; and

c) growing said altered strain under growth conditions suitable for expression of said protein.

39. The method of Claim 38, further comprising the step of collecting said protein expressed by said altered strain.

40. The method of Claim 38, wherein said host cell is selected from the group consisting of *Escherichia* and *Bacillus* cells.

41. The method of Claim 38, further comprising the step of inactivating at least one chromosomal gene selected from the group consisting of *sbo*, *slr*, *ybcO*, *csn*, *spoIISA*, *sigB, phrC, rapA, CssS trpA, trpB, trpC, trpD, trpE, trpF, tdh/kbl, alsD, sigD, prpC*, *gapB, fbp, rocA, ycgN, ycgM, rocF,* and *rocD.*

42. The method of Claim 38, wherein said protein is selected from the group consisting of homologous proteins and heterologous proteins.

43. The method of Claim 38, wherein said at least one chromosomal gene is inactivated by insertional inactivation.

44. The method of Claim 38, wherein said at least one protein is selected from the group consisting of proteases, cellulases, amylases, carbohydrases, lipases, isomerases, transferases, kinases, phosphatases, antibodies, hormones, and growth factors.

45. A method for obtaining an altered *Bacillus* strain expressing a protein of interest, comprising the steps of transforming a *Bacillus* host cell with a DNA construct comprising an incoming sequence which comprises the *pckA* gene, wherein said incoming sequence is integrated into the chromosome of said *Bacillus* host cell to produce an altered *Bacillus* strain, further in which one or more chromosomal genes have been inactivated; and growing said altered *Bacillus* strain under suitable growth conditions for the expression of a at least one protein of interest.

46. The method of Claim 45, wherein said least one protein of interest is selected from proteases, cellulases, amylases, carbohydrases, lipases, isomerases, transferases, kinases, phosphatases, antibodies, hormones, and growth factors.

47. The method of Claim 45, wherein said *Bacillus* host cell is selected from the group consisting of *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilus*, *B. thuringiensis*, *B. clausii*, *B. megaterium*, and *B. subtilis*.

48. The method of Claim 45, wherein said *Bacillus* host cell is a recombinant host cell.

49. The method of Claim 45, wherein said protein of interest produced by said altered *Bacillus* strain is recovered.

50. The method of Claim 45, wherein said DNA construct further comprises a selection marker.

51. The method of Claim 50, wherein said selection marker is excised from said incoming sequence upon integration of said incoming sequence into said chromosome of said *Bacillus* host cell.

52. The method of Claim 45, wherein the incoming sequence further comprises at lease one gene selected from the group consisting of *sbo*, *slr*, *ybcO*, *csn*, *spoIISA*, *sigB*, *phrC*, *rapA*, *CssS*, *trpA*, *trpB*, *trpC*, *trpD*, *trpE*, *trpF*, *tdh/kbl*, *alsD*, *sigD*, *prpC*, *gapB*, *fbp*, *rocA*, *ycgN*, *ycgM*, *rocF*, and *rocD*, wherein the DNA construct is integrated into the chromosome of the *Bacillus* host cell and results in the deletion of one or more genes present on said chromosome of said *Bacillus* host cell.

53. The method of Claim 45, wherein said DNA construct comprises at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:37, SEQ ID NO:25, SEQ ID NO:21, SEQ ID NO:50, SEQ ID NO:29, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:19, SEQ ID NO:31, SEQ ID NO:48, SEQ ID NO:46, SEQ ID NO:35, and SEQ ID NO:33.

54. The method of Claim 45, wherein said DNA construct comprises at least one gene that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:38, SEQ ID NO:26, SEQ ID NO:22, SEQ ID NO:57, SEQ ID NO:30, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:55, SEQ ID NO:53, SEQ ID NO:36, and SEQ ID NO:34.

55. A method for obtaining *Bacillus subtilis* strains having enhanced protease production comprising the steps of: transforming a *B. subtilis* host cell with a DNA construct; allowing homologous recombination of the DNA construct and a homologous region of the *B. subtilis* chromosome wherein *pckA* is deleted from the *B. subtilis* chromosome to produce an altered *Bacillus subtilis* strain; and growing the altered *B. subtilis* strain under conditions suitable for the expression of said protease.

56. The method of Claim 55, wherein at least one gene selected from the group consisting of *sbo*, *slr*, *ybcO*, *csn*, *spoIISA*, *sigB*, *phrC*, *rapA*, *CssS*, *trpA*, *trpB*, *trpC*, *trpD*, *trpE*, *trpF*, *tdh/kbl*, *alsD*, *sigD*, *prpC*, *gapB*, *fbp*, *rocA*, *ycgN*, *ycgM*, *rocF*, and *rocD*, is deleted from the chromosome of said *B. subtilis*.

57. The method of Claim 55, wherein said protease is selected from the group consisting of homologous proteases and heterologous proteases.

58. The method of Claim 57, wherein said protease is subtilisin.

59. The subtilisin of Claim 58, wherein said subtilisin is selected from the group consisting of subtilisin 168, subtilisin BPN', subtilisin Carlsberg, subtilisin DY, subtilisin 147, subtilisin 309, and variants thereof.

60. The method of Claim 55, wherein said altered *B. subtilis* strain further comprises a mutation in at least one gene selected from the group consisting of *degU*, *degQ*, *degS*, *scoC4*, *spoIIE*, and *oppA*.

61. The method of Claim 60, wherein said mutation comprises *degU(Hy)32*.

62. The method of Claim 55, wherein said altered *B. subtilis* strain comprises deletion of one or more indigenous chromosomal regions or fragments thereof, wherein the indigenous chromosomal region comprises about 0.5 to 500 kilobases.

63. The method of Claim 55, wherein said DNA construct comprises at least one nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:37, SEQ ID NO:25, SEQ ID NO:21, SEQ ID NO:50, SEQ ID NO:29, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:19, SEQ ID NO:31, SEQ ID NO:48, SEQ ID NO:46, SEQ ID NO:35, and SEQ ID NO:33.

64. The method of Claim 55, wherein said DNA construct comprises at least one gene that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:38, SEQ ID NO:26, SEQ ID NO:22, SEQ ID NO:57, SEQ ID NO:30, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:55, SEQ ID NO:53, SEQ ID NO:36, and SEQ ID NO:34.

65. A method for enhancing the expression of a protein of interest in *Bacillus* comprising: introducing a DNA construct including a selective marker and an inactivating chromosomal segment into a *Bacillus* host strain, wherein said DNA construct is integrated into the chromosome of the *Bacillus* host strain, resulting in the deletion of an indigenous chromosomal region or fragment thereof from said *Bacillus* host strain to produce an altered *Bacillus* strain; and growing said altered *Bacillus* strain under suitable conditions, wherein expression of a protein of interest is greater in said altered *Bacillus* strain compared to the expression of the protein of interest in said *Bacillus* host strain.

66. The method of Claim 65, further comprising the step of recovering said protein of interest.

67. The method of Claim 65, further comprising the step of excising said selective marker from said altered *Bacillus* strain.

68. The method of Claim 65, wherein said indigenous chromosomal region is selected from the group of regions consisting of PBSX, skin, prophage 7, SPβ, prophage 1, prophage 2, prophage 3, prophage 4, prophage 5, prophage 6, PPS, PKS, YVFF-YVEK, DHB, and fragments thereof.

69. The method of Claim 65, wherein said altered *Bacillus* strain comprises deletion of at least two indigenous chromosomal regions.

70. The method of Claim 65, wherein said protein of interest is selected from the group consisting of homologous proteins and heterologous proteins.

71. The method of Claim 70, wherein said protein of interest is selected from proteases, cellulases, amylases, carbohydrases, lipases, isomerases, transferases, kinases phosphatases, antibodies, hormones, and growth factors.

72. The method of Claim 65, wherein said *Bacillus* host strain is selected from the group consisting of *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens, B. brevis*, *B. stearothermophilus*, *B. clausii, B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilus,* and *B. thuringiensis*.

73. The method of Claim 65, wherein said altered *Bacillus* strain further comprises at least one mutation in a gene selected from the group consisting of *degU*, *degQ*, *degS*, *sco4*, *spoIIE* and *oppA*.

74. The method of Claim 73, wherein said mutation comprises *degU(Hy)32.*

75. A method for enhancing the expression of a protein of interest in *Bacillus* comprising: obtaining nucleic acid from at least one *Bacillus* cell; performing transcriptome DNA array analysis on the nucleic acid from said *Bacillus* cell to identify at least one gene of interest; modifying said at least one gene of interest to produce a DNA construct; introducing said DNA construct into a *Bacillus* host cell to produce an altered *Bacillus* strain, wherein said altered *Bacillus* strain is capable of producing a protein of interest, under conditions such that expression of said protein of interest is enhanced as compared to the expression of the protein of interest in a *Bacillus* that has not been altered.

76. The method of Claim 75, wherein said protein of interest is associated with at least one biochemical pathway selected from the group consisting of amino acid biosynthetic pathways and biodegradative pathways.

77. The method of Claim 76, wherein at least one biodegradative pathway is disabled.

78. The method of Claim 77, wherein said biodegradative pathway is disabled due to the transcription of a gene of interest.

79. The method of Claim 75, wherein said *Bacillus* host cell is selected from the group consisting of *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens, B. brevis*, *B. stearothermophilus*, *B. clausii, B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilus* and *B. thuringiensis*.

80. The method of Claim 75, wherein said protein of interest is selected from the group consisting of homologous proteins and heterologous proteins.

81. The method of Claim 80, wherein said protein of interest is selected from proteases, cellulases, amylases, carbohydrases, lipases, isomerases, transferases, kinases, phosphatases, antibodies, hormones, and growth factors.

82. A method for enhancing the expression of a protein of interest in *Bacillus*, comprising: obtaining nucleic acid containing at least one gene of interest from at least one *Bacillus* cell; fragmenting said nucleic acid; amplifying said fragments to produce a pool of amplified fragments comprising said at least one gene of interest; ligating said amplified fragments to produce a DNA construct; directly transforming said DNA construct into a *Bacillus* host cell to produce an altered *Bacillus* strain, wherein said altered Bacillus strain comprises a modified gene selected from the group consisting of *prpC*, *sigD* and *tdh/kbl*; culturing said altered *Bacillus* strain under conditions such that expression of said protein of interest is enhanced as compared to the expression of said protein of interest in a *Bacillus* that has not been altered.

83. The method of Claim 82, wherein said *Bacillus* host cell is selected from the group consisting of *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens*, *B. brevis*, *B. stearothermophilus*, *B. clausii*, *B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilus* and *B. thuringiensis*.

84. The method of Claim 82, wherein said protein of interest is selected from the group consisting of homologous proteins and heterologous proteins.

85. The method of Claim 84, wherein said protein of interest is selected from proteases, cellulases, amylases, carbohydrases, lipases, isomerases, transferases, kinases, phosphatases, antibodies, hormones, and growth factors.